Journal of King Saud University - Science 36 (2024) 103238

HOSTED BY

Contents lists available at ScienceDirect

Journal of King Saud University - Science

journal homepage: www.sciencedirect.com

Full Length Article

Exploring the therapeutic potential of *Cynanchum tunicatum* (Retz.) Alston-assessment of phytochemicals and biological activities

Deepika Krishnamoorthy [a], Amutha Swaminathan [a,*], Amal Mohamed AlGarawi [b], Lavanya Nallasamy [a], Girija Sangari Murugavelu [a], Swarna Lakshmi Selvaraj [a]

[a] *Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India*
[b] *Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*

ARTICLE INFO

Keywords:
Cynanchum tunicatum
Antioxidant activity
Anti-inflammatory activity
And phytochemical analysis

ABSTRACT

Cynanchum tunicatum (Retz.) Alston is native to the Asian region and is distributed in the tropical areas of India and Sri Lanka. The aim of this study is to explore the phytochemical composition, antioxidant potential, and anti-inflammatory properties of *C. tunicatum*. Metabolic profiling was carried out using phytochemical screening to detect and quantify the secondary metabolites. To evaluate the potential secondary metabolites using the standard methods, Fourier transform infrared (FTIR), High-Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography–Mass Spectrometry (GC–MS) from organic extracts of *C. tunicatum* and its biological activities. FTIR investigated peaks that represent alkane and aromatic compounds. GC–MS revealed the presence of 22 constituents such as 1-Hexacosene (0.145 %), l-(+)-Ascorbic acid 2,6-dihexadecanoate (8.129 %), Campesterol (5.243 %), Beta −Amyrin (10.614 %), Lupeol (13.061 %), Octadecanoic acid (0.751 %) are the major active compounds present. HPTLC fingerprinting confirms the bioactive compounds such as colchicine, strychnine, coumarin etc. which are represented with corresponding R_f values. Among all the extracts of *C. tunicatum* methanolic extract showed highest antioxidant activities. In 2,2-diphenylpicrylhydrazyl method exhibit the IC_{50} of (38.91 μg/mL), Ferric reducing antioxidant power assay (1.6 μg/mL), total antioxidant assay (IC_{50} = 32.91 μg/mL) and IC_{50} of anti-inflammatory activity (42.31 μg/mL) respectively. These findings enrich the knowledge of the species *Cynanchum tunicatum* for the possible application as a source of bioactive compounds in drug discovery.

1. Introduction

Medicinal plants are employed in the treatment of a wide array of diseases and serve as abundant sources of secondary metabolites. Folklore medicine has been used from ancient period in several countries (Sridharan et al., 2022). In rural communities, traditional medicine practitioners were used for many diseases (Archana & Bose, 2022). The secondary metabolites significantly shapes the biological and pharmacological efficacy of the product, consequently posing substantial challenges to achieving reproducibility in preclinical investigations and clinical trials (Alcazar Magana et al., 2020).

Cynanchum tunicatum belongs to the family Apocynaceae, which comprises approximately 300 species, including some types of swallowworts. Most of these species are climbers or twiners. In Chinese medicine, different varieties of *Cynanchum* species has their essential phytochemical constituents are prescribed to treat fever, cough, pneumonia and asthma (Bailly et al., 2023). Recent pharmacological studies have demonstrated that *Cynanchum* plants possess significant pharmacological effects, such as anti-oxidation, immune regulation, anti-inflammatory, and anti-tumor. A literature survey shows that approximately 450 compounds have been isolated from various *Cynanchum* species (Han et al., 2018). *Cynanchum* species harbor a myriad of therapeutic compounds; however, so far, researchers have not explored the biological activity of the specific plant species *C. tunicatum*.

A number of compounds such as β-sitosterol, conduritol F, geniposide, wilfoside was identified in *C. wilfordii* and *C. auriculatum*. Literature survey evidences that the most important lacuna of bioactive compounds present in the *C. tunicatum*. The primary objective of this study is to explore the bioactive compounds and their biological properties. Fingerprinting of *C. tunicatum* was conducted using Gas Chromatography-Mass Spectrometry (GC–MS) and Fourier Transform Infrared (FTIR) spectroscopy. Additionally, High-Performance Thin

* Corresponding author.
E-mail address: dr.amuthaswaminathan@gmail.com (A. Swaminathan).

https://doi.org/10.1016/j.jksus.2024.103238
Received 5 January 2024; Received in revised form 3 May 2024; Accepted 5 May 2024
Available online 7 May 2024

Layer Chromatography (HPTLC) was employed as an effective technique for metabolite profiling.

2. Materials and methods

2.1. Plant material

The plant *C. tunicatum* was collected from Sirumalai forest, Dindigul, Tamil Nadu (N:10°16′45.1; E: 77 °59′55.1). The plant is maintaining in Herbal Garden, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. The Plant was authenticated by Botanical Survey of India, Southern Region, Coimbatore (BSI/SRC/5/23/2023/Tech-551).

2.2. Solvents, chemicals, and extraction

Hexane, chloroform, ethyl acetate, methanol, toluene, acetone, formic acid, Mayer's reagent Wagner's reagent, Drangendorff's reagent, Molisch's reagent, Fehling reagent, Barfoed reagent, Bendict's reagent, Borntrager's reagent, sulphuric acid, ammonium solution, copper sulphate, sodium nitroprusside, ninhydrin, gallic acid, atropine, rutin, gallic acid and linalool, diclofenac sodium, DPPH reagent, Ascorbic acid, FRAP reagent, sulfuric acid, sodium phosphate, ammonium molybdate, Bovine serum albumin were used for further analysis. Atropine, Rutin, Ascorbic acid, Linalool from Sigma-Aldrich and other reagents were purchased from Sisco Research Laboratories.

Fresh and healthy plants were rinsed, shade-dried, and then pulverized. The extracts were obtained through sequential extraction of phytochemicals, transitioning from non-polar to polar solvents, employing hexane, chloroform, ethyl acetate, and methanol. All the crude extracts were dried using rotary vacuum evaporator (Roteva) and dissolved with respective solvents at mg/mL concentrations for further analysis.

2.3. Qualitative and quantitative phytochemical screening

The preliminary qualitative phytochemicals were analysed in all four extracts of *C. tunicatum* to determine the secondary metabolites (Harborne, 1998; Raaman, 2006). The Quantitative analysis of Alkaloids, Flavonoids, Phenols and Terpenoids were estimated by the following methods.

2.3.1. Total alkaloid content of C. tunicatum

The estimation of alkaloids was determined by bromocresol green method (Ajanal et al., 2012) for all four extracts of *C. tunicatum*. The extracts were dissolved in 2 N HCl (pH 2) and subsequently filtered. The resulting 10 mL solution was transferred to a separatory funnel and subjected to three washes with 50 mL of chloroform each. Following this, 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. Shake the mixture and extract yellow-coloured complex with 1-, 2-, 3- and 4-mL of chloroform in a separating funnel and collect the extract in 10 mL volumetric flask make up with chloroform. Atropine is used as a standard with different concentrations (0.2, 0.4, 0.6, 0.8 and 1 µg/ mL). The absorbance was measured using a spectrophotometer at 470 nm.

2.3.2. Total flavonoid content of C. tunicatum

The aluminium chloride method (Slinkard & Singleton, 1977) was used for flavonoid estimation using various extracts of *C. tunicatum*. Take 0.5 mL of all four extracts and mixed with 0.1 mL of 10 % aluminium chloride, 0.1 mL of potassium acetate and 2.8 mL of distilled water. It incubates at room temperature for 30 min. Rutin was used as a standard with various concentrations (0.2, 0.4, 0.6, 0.8, 1 µg/mL). The absorbance of the reaction mixture was measured at 415 nm.

2.3.3. Total Phenol content of C. tunicatum

Total phenolics were determined by Folin Ciocalteau (Slinkard & Singleton, 1977), using various extracts of *C. tunicatum*. One mL of extract was added with 1.0 mL of Folin Ciocalteau reagent followed by the addition of 3.0 mL of 2 % sodium carbonate and the mixture kept for 2 hrs incubation in dark condition. Gallic acid used as a standard. The blue colour indicates the presence of phenols and the absorbance was measured at 760 nm.

2.3.4. Determination of total terpenoid content of Cynanchum tunicatum

The total terpenoid content was determined by colorimetry assay (Indumathi et al., 2014) using various extracts of *C. tunicatum*. One mL of plant extract was mixed with 150 µL of vanillin-glacial acetic acid solution (5 %) followed by the addition of 500 µL of perchloric acid. The mixture was kept under water bath for 45 mins at 60℃. Glacial acetic acid (2.25 mL) was added to the mixture and observed reddish pink to blue color. The absorbance was measured at 548 nm. Linalool is used as a standard.

2.4. Determination of antioxidant activity

2.4.1. DPPH assay of C. tunicatum

The DPPH free radical scavenging activity was assessed according to the method described by Hatano et al. (1988). All four extracts of *C. tunicatum* at various concentrations (20, 40, 60, 80, 100 µg/mL) were combined with 2.7 mL of a 0.1 mM methanolic solution of DPPH. The resulting mixture was vigorously shaken and allowed to incubate in the dark for 30 min at room temperature. Subsequently, the absorbance was measured at 517 nm. A control sample, devoid of any extract but containing DPPH, was also measured. The percentage inhibition and IC_{50} value were then calculated. The absorbance of positive control is a pure compound. Hence it is showed higher inhibition percentage than plant crude extracts.

2.4.2. FRAP − assay of C. tunicatum

The capacity to reduce ferric ions was evaluated using a modified protocol based on the method outlined by Benzie & Strain, (1996). A portion of each extract, spanning various concentrations (20, 40, 60, 80, 100 µg/mL), was combined with 3 mL of FRAP reagent composed of 10 parts of 300 mM sodium acetate buffer (pH 3.6), 1 part of 10 mM 2,4,6-Tripyridyl-S-Triazine solution, and 1 part of 20 mM FeCl3 6H2O solution. The reaction mixture was then incubated in a water bath at 37℃ for 30 min. Gallic acid served as the standard reference. Subsequently, the absorbance was recorded at 593 nm, and the FRAP value was computed.

2.4.3. Total antioxidant activity of C. tunicatum

The total antioxidant capacity of *C. tunicatum* was assessed using the phosphomolybdenum method as described by (Prieto et al., 1999). A 0.5 mL aliquot of each of the four extracts, prepared at various concentrations (20, 40, 60, 80, 100 µg/mL), was combined with 4.5 mL of phosphomolybdenum reagent, consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixtures were then incubated in a water bath at 95℃ for 90 min. Ascorbic acid served as the standard reference. Subsequently, the absorbance was measured at 695 nm, and the inhibition percentage and IC_{50} value were determined.

2.5. Evaluation of anti-inflammatory activity

The anti-inflammatory activity of all four extracts of *C. tunicatum* was assessed using the bovine serum albumin denaturation assay, as described by Chandra et al., (2012). A reaction mixture of 5 mL was prepared, comprising 0.2 mL of bovine serum albumin, 2.8 mL of phosphate-buffered saline (pH 6.4), and 2 mL of various concentrations (20, 40, 60, 80, 100 µg/mL) of plant extracts. The mixtures were then

incubated at (37 ± 2) ℃ in a Biochemical Oxygen Demand (BOD) incubator for 15 min, followed by heating at 70℃ for 5 min. Subsequently, the absorbance was measured at 660 nm, with diclofenac sodium serving as the standard reference.

Percentage inhibition (%) = [Sample absorbance/(Control absorbance − 1)] × 100

2.6. FTIR (Fourier transform infrared) analysis

FTIR (Shimadzu Miracle 10) equipped with a temperature-stabilized detector was used for the analysis of functional groups present in phytochemicals in the range of 400–4000 cm^{-1} with a resolution of 16 cm^{-1}. The characteristic peaks and their functional groups were detected and recorded for further structure elucidated using FTIR.

2.7. GC–MS analysis

Mass spectrometry data were utilized in conjunction with GC–MS/MS analysis to identify compounds present within the extract. Interpretation of mass spectra was facilitated using the comprehensive database provided by the National Institute of Standards and Technology (NIST), which encompasses over 62,000 patterns. Unknown components were matched against known compounds stored within the NIST library. The identification process involved determination of the name, molecular weight, and molecular formula of the extract samples (Valdez et al., 2018).

2.8. HPTLC fingerprinting analysis

HPTLC profiling is used for the separation, identification, determination, and validation of phytoconstituents such as alkaloids, flavonoids, phenols and terpenoids from methanolic extracts of *C. tunicatum*. Aliquot of the test solution (100 mg / mL) and the standard solution were loaded as 5 mm band length in a silica gel 60F254TLC plate using a Hamilton syringe and LINOMAT 5 instrument (CAMAG, Muttenz, Switzerland). The samples were loaded in TLC plate with respective mobile phases and the images were captured in white light and UV light at 254 nm and 366 nm of developed and derivatized plates.

2.9. Statistical analysis

The experimental results were expressed as mean ± standard deviation. Statistical significance was evaluated by one-way analysis of variance (ANOVA). Values of $p \leq 0.05$ were significant using Tukey's test.

3. Results and discussion

3.1. Phytochemical analysis

The preliminary qualitative analysis was performed in four different extracts of *C. tunicatum*. The secondary metabolites are rich in ethyl acetate and methanolic extracts such as alkaloids, carbohydrates and glycosides, phenol and terpenoids, quinine, and phytosteroids (Table 1). Similar results were obtained in genus *Cynanchum* such as steroids, saponins, alkaloids, flavonoids, phenols and terpenoids (Han et al., 2018). According to Shrivastava et al., analyzed the terpenoids, flavonoids, tannins, saponins, steroids, carbohydrates, and alkaloids in methanolic fruit extract of *Trichosanthes dioica* (Shrivastava et al., 2021). In *Ricinus communis,* Rahman et al. studied the plant secondary metabolites in seed extract such as terpenoids, flavonoids, coumarin, steroids, and reducing sugar. These secondary metabolites are exclusively significant for the plant defence mechanism (Rahman et al., 2022).

Table 1
Preliminary qualitative phytochemical screening of *Cynanchum tunicatum.*

S. No	Tests	Hexane	Chloroform	Ethyl acetate	Methanol
1	**Alkaloids**				
A	Mayer's Test	−	−	++	++
B	Wagner's Test	++	−	+	+
C	Drangendorff's Test	+	−	−	+
2	Flavonoids	+	−	++	++
3	**Carbohydrates and glycosides**				
A	Molisch's Test	++	++	+	+
B	Fehling Test	−	+	++	+
C	Barfoed Test	+	+	++	++
D	Bendict's Test	−	−	++	++
E	Borntrager's Test	+	+	+	++
4	Saponin Test	−	−	+	+
5	**Oils and fats test**				
A	Spot Test	−	−	−	+
6	**Phenolic and terpenoid test**				
A	Ferric chloride Test	−	−	+	++
B	Gelatin Test	−	−	−	++
C	Lead Acetate Test	++	−	−	++
D	Alkaline Reagent Test	−	−	++	++
E	Magnesium and Hydrochloric Acid Test	+	−	−	++
F	Phlobaterpenoids	−	−	−	−
7	Quinone	++	−	++	+
8	Glycosides	++	−	++	++
9	Cardiac glycosides	+	−	−	−
10	Terpenoid	++	−	+	+
11	Coumarins	−	−	−	+
12	Steroids	−	−	+	+
13	Phytosterols	−	++	++	++
14	Protein	+	−	++	+

−: Absent, +: Present, ++: Strongly Present.

3.1.1. Estimation of total alkaloid content of Cynanchum tunicatum

Alkaloids are specific remedy for many diseases particularly in mammals because of their general toxicity, deterrence capability, adaptogenic activities and anti-inflammatory activity which help to alleviate pain endurance against stress and resistance to diseases (Zhang & Hu, 2020). In *C. tunicatum* the alkaloid contents were quantified in all the extract. Interestingly, the maximum alkaloid content was found in methanolic extract (1.37 ± 0.03 µg/mL) followed by ethyl acetate extract (0.78 ± 0.05 µg/mL), hexane (0.67 ± 0.03 µg/mL), chloroform (0.53 ± 0.04 µg/mL) of *C. tunicatum* (Fig. 1(e)). Atropine is used as a reference standard (Fig. 1(a)). Nagalakshmi et al. also studied that the maximum alkaloid content (5.62 mg/g) in methanolic extract of *Tinospora cordifolia* was observed (Nagalakshmi et al., 2023).

3.1.2. Total flavonoid content of C. tunicatum

The total flavonoid content was determined using aluminium chloride method of various solvents. The yellow colour represented the presence of flavonoid. The maximum flavonoid content was observed in methanolic extracts (4.23 ± 0.05 µg/mL) followed by ethyl acetate (2.37 ± 0.01 µg/mL), chloroform (1.28 ± 0.02 µg/mL) and hexane (0.104 ± 0.01 µg/mL) (Fig. 1(e)). Rutin is used for a reference standard (Fig. 1 (b)). Interestingly, Garg & Garg, analysed flavonoid content in methanolic leaf extract of *Ocimum sanctum,* obtained the maximum amount (4.75 mg /100 mg) and it is correlated with antioxidant activity (Garg & Garg, 2019).

3.1.3. Total Phenol content of C. tunicatum

The Phenolic content of hexane, chloroform, ethyl acetate and methanolic extracts of *C. tunicatum* showed 1.33 ± 0.01 µg/mL, 1.35 ± 0.007 µg/mL, 2.27 ± 0.063 µg/mL and 2.32 ± 0.037 µg/mL respectively, in which methanolic extract showed the maximum amounts of phenols (Fig. 1 (e)). Gallic acid is used as a reference standard (Fig. 1(c)).

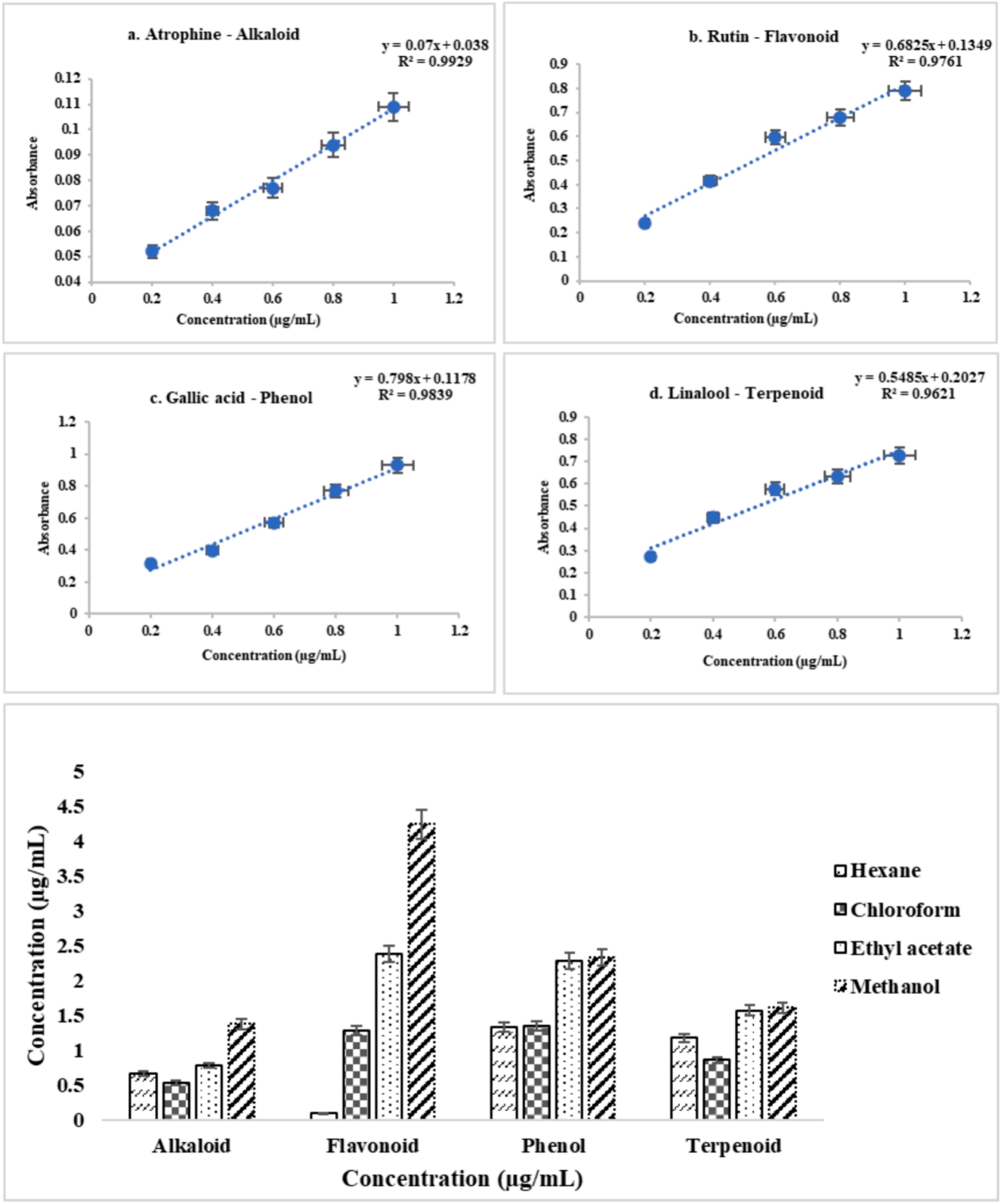

Fig. 1. a) Calibration curve of standard atropine for the quantification of total alkaloid content b) Calibration curve of standard rutin for the quantification of total flavonoid content c) Calibration curve of standard gallic acid for the quantification of total phenol content d) Calibration curve of standard linalool for the quantification of total terpenoid content e) Graphical representation of quantitative secondary metabolite screening of *Cynanchum tunicatum*.

Similarly, the previous researchers also analyzed the flavonoid content in various medicinal plants. Arya et al. studied the methanolic leaf extract of *Cichorium intybus* containing maximum phenolic content (302 ± 0.251 mg/mL) (Arya et al., 2022). Ahmed et al. investigated the methanolic extract of *Cannabis sativa* encompassing the maximum phenolic content (36.42 ± 1.905 μg/mL) (Abdel-Azeem et al., 2020).

3.1.4. Total terpenoid content of C. tunicatum

Terpenoid contents were analysed using the folin–ciocalteu method using various solvents of *C. tunicatum*. The maximum content of terpenoid was observed in methanol (1.60 ± 0.02 μg/mL) followed by ethyl acetate (1.57 ± 0.14 μg/mL), hexane (1.18 ± 0.02 μg/mL) and chloroform (0.86 ± 0.02 μg/mL) (Fig. 1 (e)). Linalool is used as a standard (Fig. 1 (d)). (Indumathi et al., 2014) analysed the highest terpenoid content in the methanolic leaf extract of *Enicostemma litorrale*. Terpenoids are significantly helpful for therapeutic properties like anticancer, antimicrobial and antioxidant activity (Roaa, 2020).

Preliminary phytochemical investigation of *C. tunicatum* facilitates the identification of phytochemical constituents and is quantified in the ranked as flavonoids > phenols > terpenoids > alkaloid. The differences in the values were statistically significant ($p \leq 0.05$). Based on the previous literature (Wang et al., 2021), *Cynanchum* species contains 232 compounds including alkaloids, terpenoids, C_{21} steroids, flavonoids, acetophenones and these phytochemical compounds are abundantly present in *C. tunicatum*. In future, based on the secondary metabolites biological activities will be carried out to confirm the medicinal properties of the *C. tunicatum*.

3.2. Antioxidant assays

Reactive oxygen species (ROS) damage the cells through pollution, radiations, and other environmental factors. These damages were protected through antioxidants which are the molecules that can neutralize the ROS by donating the electrons and stabilizes hazardous free radicals. The mechanisms of antioxidant activity either directly or indirectly by inhibiting the free radical damage, thereby preventing the nucleic acids, proteins, lipids, and other molecules. Plant derived bioactive compounds protect the cells from free radical damage by the free radical scavenging activity. In this present study, bioactive compounds obtained from *Cynanchum tunicatum* extracts showed more efficient antioxidant potential was analysed by DPPH, FRAP, and TAA.

3.2.1. DPPH assay of C. tunicatum

Free radicals cause damage to the plant cells, whereas antioxidants play a crucial role in protecting the cells. Natural antioxidants such as phenols and flavonoids have the potential to provide resistance against free radical-induced oxidative stress (Karale et al., 2022). The antioxidant activity was anlayzed for all four crude extracts of *C. tunicatum* using DPPH assay. The maximum inhibition percentage was obtained in the methanolic extract (IC_{50} = 38.91 μg/mL) followed by ethyl acetate (IC_{50} = 52.26 μg/mL) (Table 2). Ascorbic acid was used as a reference standard and its IC_{50} value is 53.101 μg/mL. This result confirmed the methanolic extract of *C. tunicatum* have potential for antioxidant activity. Still there is no previous antioxidant study were found on *C. tunicatum*. Akgül et al. reported that Ethyl acetate extract showed maximum inhibition (68.721 ± 1.694) of free radical scavenging activity in *Euphorbia eriophora* (Akgül et al., 2022).

3.2.2. FRAP assay of C. tunicatum

The determination of antioxidant activity, based on their ferric-reducing power, involves assessing the ability to reduce Fe_3 + to Fe_2+ (Ene-Obong et al., 2018). The antioxidant activity of all four crude extracts of *C. tunicatum* by using ferrous reducing assay. The maximum reduction was observed at a concentration of 100 μg/mL in methanolic extract (1.6 ± 0.11) followed by ethyl acetate (1.2 ± 0.007), chloroform (1.09 ± 0.009) and hexane (0.98 ± 0.007) (Table 3). The ferric-reducing assay of methanolic extract shows higher antioxidant capacity compared to gallic acid. According to Noreen et al. reported that the ethanolic extract of *Coronopus didymus* (aerial parts) observed the highest Optical Density (OD) value is 0.304 at the concentration of 50 μg/mL (Noreen et al., 2017).

3.2.3. Total antioxidant activity of C. tunicatum

The total antioxidant activity is based on the phosphomolybdate reagent which reduces Molybdenum VI to V with maximum absorption and it forms a green phosphate/Mo (V) complex. All four extracts of *C. tunicatum* with various concentrations were analysed and the maximum activity was observed at 100 μg/mL in methanol extract (IC_{50} = 32.91 μg/mL) followed by ethyl acetate (IC_{50} = 47.11 μg/mL), chloroform (IC_{50} = 86 μg/mL) and hexane (IC_{50} = 82.11 μg/mL) (Table 4). Hence the *C. tunicatum* is a potential candidate of antioxidant property. Ascorbic acid was used as a standard (IC_{50} = 53.38 μg/mL). In the previous research evidenced that the ethanolic leaf extract of *Limonia acidissima* showed maximum antioxidant activity of 5.055 μL (Parvez & Sarker, 2021). Bayliak *et al.*, studied that highest reducing ability of

Table 3
Evaluation of Ferric Reducing Antioxidant Power (FRAP) of *Cynanchum tunicatum*.

Concentration (μg/mL)	FRAP value			
	Hexane	Chloroform	Ethyl acetate	Methanol
20	0.02 ± 0.0005 ^{e}	0.24 ± 0.009 ^{e}	0.11 ± 0.005 ^{e}	0.29 ± 0.01 ^{e}
40	0.16 ± 0.01 ^{d}	0.43 ± 0.01 ^{d}	0.40 ± 0.01 ^{d}	0.45 ± 0.009 ^{d}
60	0.44 ± 0.01^{c}	0.59 ± 0.01^{c}	0.63 ± 0.02^{c}	0.89 ± 0.03^{c}
80	0.65 ± 0.004^{b}	0.84 ± 0.001^{b}	1.008 ± 0.009^{b}	1.20 ± 0.01^{b}
100	0.98 ± 0.007 ^{a}	1.09 ± 0.009 ^{a}	1.20 ± 0.007 ^{a}	1.60 ± 0.11 ^{a}

All values were expressed in mean ± standard deviations of triplicate measures. Each analysis was statistically significant ($p \leq 0.05$), 'a' expressed best results and 'e' showed poor results.

Table 2
Evaluation of Radical Scavenging activity of *Cynanchum tunicatum* by using DPPH method.

Concentration (μg/mL)	Percent scavenging of stable DPPH free radical (%)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Ascorbic acid
20	8.67 ± 0.003 ^{e}	26.4 ± 0.01 ^{e}	29.3 ± 0.004 ^{e}	40.3 ± 0.007 ^{e}	22.76 ± 0.004 ^{e}
40	20.7 ± 0.003 ^{d}	36.4 ± 0.006 ^{d}	39.4 ± 0.009 ^{d}	52.09 ± 0.004 ^{d}	40.4 ± 0.004 ^{d}
60	35.01 ± 0.002^{c}	48.4 ± 0.005^{c}	55.6 ± 0.002^{c}	57.9 ± 0.01^{c}	56.05 ± 0.007^{c}
80	54.1 ± 0.008^{b}	58.3 ± 0.005^{b}	68.1 ± 0.005^{b}	72.5 ± 0.004^{b}	71.39 ± 0.006^{b}
100	69.1 ± 0.004^{a}	70.8 ± 0.01 ^{a}	84.08 ± 0.01 ^{a}	85.9 ± 0.005 ^{a}	86.82 ± 0.002 ^{a}
IC_{50}	76.15	63.405	52.26	38.91	53.101

All values were expressed in mean ± standard deviations of triplicate measures. Each analysis was statistically significant ($p \leq 0.05$), 'a' expressed best results and 'e' showed poor results.

Table 4
Evaluation of Total antioxidant activity of *Cynanchum tunicatum* by phosphomolybdenum method.

Concentration (µg/mL)	Percent scavenging of TAA (%)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Ascorbic acid
20	13.6 ± 0.03 [e]	15.4 ± 0.07 [e]	29.9 ± 0.01 [e]	44.2 ± 0.005 [e]	27.3 ± 0.02 [e]
40	21.1 ± 0.023 [d]	17.5 ± 0.047 [d]	48.0 ± 0.008 [d]	54.1 ± 0.008 [d]	44 ± 0.02 [d]
60	30.0 ± 0.003[c]	31.2 ± 0.027[c]	60.3 ± 0.007[c]	62.4 ± 0.007[c]	53.1 ± 0.01[c]
80	57.0 ± 0.013[b]	45.1 ± 0.057[b]	70.3 ± 0.009[b]	75.1 ± 0.002[b]	68.1 ± 0.02[b]
100	58.6 ± 0.023 [a]	62.1 ± 0.017 [a]	81.8 ± 0.006 [a]	86.3 ± 0.003 [a]	83.6 ± 0.002 [a]

All values were expressed in mean ± standard deviations of triplicate measures. Each analysis was statistically significant ($p \leq 0.05$), 'a' expressed best results and 'e' showed poor results.

Rhodiola rosea aqueous extract was 3.66 ± 0.24 mg/mL (Bayliak et al., 2016).

3.3. Evaluation of anti-inflammatory activity assay

Protein is denatured by harmful pathogens or free radicals and inhibiting this process can be a potential mechanism for anti-inflammatory activity (Yesmin et al., 2020). Result displayed in Table 5 show IC_{50} inhibition of albumin denaturation of all the extracts of *C. tunicatum* at concentration between 42.31 to 68.44 µg/mL in the following order of methanol > ethyl acetate > hexane > chloroform. The standard diclofenac sodium an anti-inflammatory drug showed IC_{50} value at 60.14 µg/mL. According to *Phoenix dactylifera* (Jihl − a variety of date seed) showed a high inhibition (IC_{50} = 90.34 µg/mL) in scavenging nitric oxide free radicals and possessed the highest anti-denaturation effect (Hmidani et al., 2020). Similarly, Saleem et al. analysed BSA assay in *Moringa oleifera* showed maximum inhibition percentage of protein denaturation with IC_{50} value of butanol > n-hexane > ethyl acetate > piroxicam > diclofenac sodium > methanolic > aqueous (Saleem et al., 2020). In this present study, the methanolic extract contains alkaloids, flavonoids, phenols and terpenoids are the main factors influenced the anti-inflammatory activity.

3.4. FTIR analysis of C. tunicatum

FTIR spectrum of whole plant of *C. tunicatum* from hexane, chloroform, ethyl acetate and methanol crude extracts were analysed to identify the functional groups. The information in Fig. 2 showed peak values along with their functional group of various extracts, respectively. The main peak observed in FTIR spectra of hexane extract at 2870.08 cm^{-1} (medium), 2924.09 cm^{-1} (strong), 2954.95 cm^{-1} (narrow sharp strong), 1728.22 cm^{-1} (weak), 1465.90 cm^{-1} (strong), 1381.03 cm^{-1} (medium), 1242.16 cm^{-1} (weak), 1172.72 cm^{-1} (weak), 1041.56 cm^{-1} (weak), 887.26 cm^{-1} (medium), 725.23 cm^{-1} (strong), 817.82 cm^{-1} (weak), 563.21 cm^{-1} (weak). Infrared spectrum of chemical constituents of chloroform exhibited peaks at 3325.28 cm^{-1} (broad strong), 2947.23 cm^{-1} (medium), 2831.50 cm^{-1} (sharp medium), 1666.50 cm^{-1} (very weak), 1404.18 cm^{-1} (weak), 1026.13 cm^{-1} (strong) and 686.66 cm^{-1} (medium). The peak of ethyl acetate represented at 3633.89 cm^{-1} (weak), 2985.81 cm^{-1} (sharp medium), 2900.94 cm^{-1} (very weak), 1735.93 cm^{-1} (sharp narrow strong), 1442.75 cm^{-1} (medium), 1373.32 cm^{-1} (sharp medium), 1234.44 cm^{-1} (sharp strong), 1095.57 cm^{-1} (medium), 1041.56 cm^{-1} (sharp narrow strong), 786.96 cm^{-1} (broad medium), 848.68 cm^{-1} (sharp medium), 632.65 cm^{-1} (medium), 609.51 cm^{-1} (medium). The FTIR spectra of methanol extract showed peaks at 3317.56 cm^{-1} (broad medium), 2831.50 cm^{-1} (sharp medium), 2939.52 cm^{-1} (medium), 2522.89 cm^{-1} (weak), 2229.71 cm^{-1} (very weak), 2044.54 cm^{-1} (weak), 1666.50 cm^{-1} (weak), 1450.47 cm^{-1} (medium), 1111.00 cm^{-1} (medium), 1018.41 cm^{-1} (narrow strong), 671.23 cm^{-1} (broad medium), 601.79 cm^{-1} (medium), 524.64 cm^{-1} (medium).

The spectra of *C. tunicatum* with the frequency range from 2831.50 to 2985.81 represented the presence of alkane group (C–H stretch). The peak at 3325.28 and 3633.89 corresponds to the presence of alcohol (O–H stretch) (Vahur et al., 2016). The peak at 2522.89 was observed as carboxylic acid (O–H stretch). The peak at 2229.71 showed the presence of nitriles (C≡N stretch). The peak at 2044.54 signified the presence of isothiocyanate (N = C = S stretch). The peak at 1666.50 and 1728.22 to 1735.93 unveiled the presence of aromatic compounds (C–H bending). The peak at 1450.47 and 1465.90 displayed alkane (O–H bending) (Reignier et al., 2021). The peak at 1404.18 and 1442.75 showed carboxylic acid (O–H bending). The peak at 1381.03 and 1373.32 exhibited alcohol (O–H bending). The peak ranges from 1172.72 to 1242.16 represented by amine (C–H stretch). The peak ranges from 1018.41 to 1041.56 showed a sulfoxide group (S = O stretch). The medium peak at 887.26 exhibited alkene (C = C bending). The peak ranges from 563.21 to 848.68 showed a halo compound (C-Cl stretch). The medium peak at 524.64 contained a halo compound (C-I stretch) (Papakosta et al., 2020). The FTIR spectrum conforms to the presence of alkaloids, flavonoids, phenols, terpenoids, carboxylic acid, alkane, aromatic alkenes in *C. tunicatum*.

3.5. GC–MS analysis

The GC–MS analyses of the methanolic extract of the whole plant of C. *tunicatum* provided the separation of 22 components, from 11 different groups were well identified using their mass spectra and retention index data. The major compounds are 1-Hexacosene (0.145 %), 17-(1,5-Dimethylhexyl)-10,13-dimethyl-2 (0.180 %), 1,3-Dioxolane, 4-methyl-2-pentadecyl (0.382 %), 7-Dehydrodiosgenin (0.155 %), Methyl Palmitoleate (0.119 %), Palmitic acid (0.988 %), Methyl (Z)-5,11,14,17-eicosatetraenoate (0.13 %), Octadecanoic acid (0.751 %), Myristic acid (0.141 %), Methyl linoleate (0.147 %), l-(+)-Ascorbic acid 2,6-dihexadecanoate (8.129 %), Campesterol (5.243 %), Phenol, 2,6-dimethoxy-4-(2-propenyl)- (0.108 %), 4.alpha.,14-Dimethyl-5. alpha-

Table 5
Evaluation of Anti-inflammatory activity of *Cynanchum tunicatum* by protein denaturation assay.

Concentration (µg/mL)	Percent inhibition of anti-inflammatory (%)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Diclofenac sodium
20	24.8 ± 0.002[e]	2.51 ± 0.0005[e]	13.22 ± 0.0005[e]	49.2 ± 0.002[e]	39.3 ± 0.003[e]
40	118.009 ± 0.002[d]	29.5 ± 0.003[d]	28.1 ± 0.003[d]	71.01 ± 0.002[d]	122.09 ± 0.01[d]
60	194.6 ± 0.001[c]	62.8 ± 0.002[c]	50.7 ± 0.002[c]	102.8 ± 0.001[c]	221.7 ± 0.007[c]
80	268.08 ± 0.003[b]	97.4 ± 0.001[b]	71.4 ± 0.001[b]	147.8 ± 0.004[b]	301.1 ± 0.004[b]
100	310.7 ± 0.002 [a]	130.8 ± 0.002 [a]	85.7 ± 0.002[a]	171.01 ± 0.001[a]	374.5 ± 0.009[a]
IC_{50}	59.23	68.44	57.03	42.31	60.14

All values were expressed in mean ± standard deviations of triplicate measures. Each analysis was statistically significant ($p \leq 0.05$), 'a' expressed best results and 'e' showed poor results.

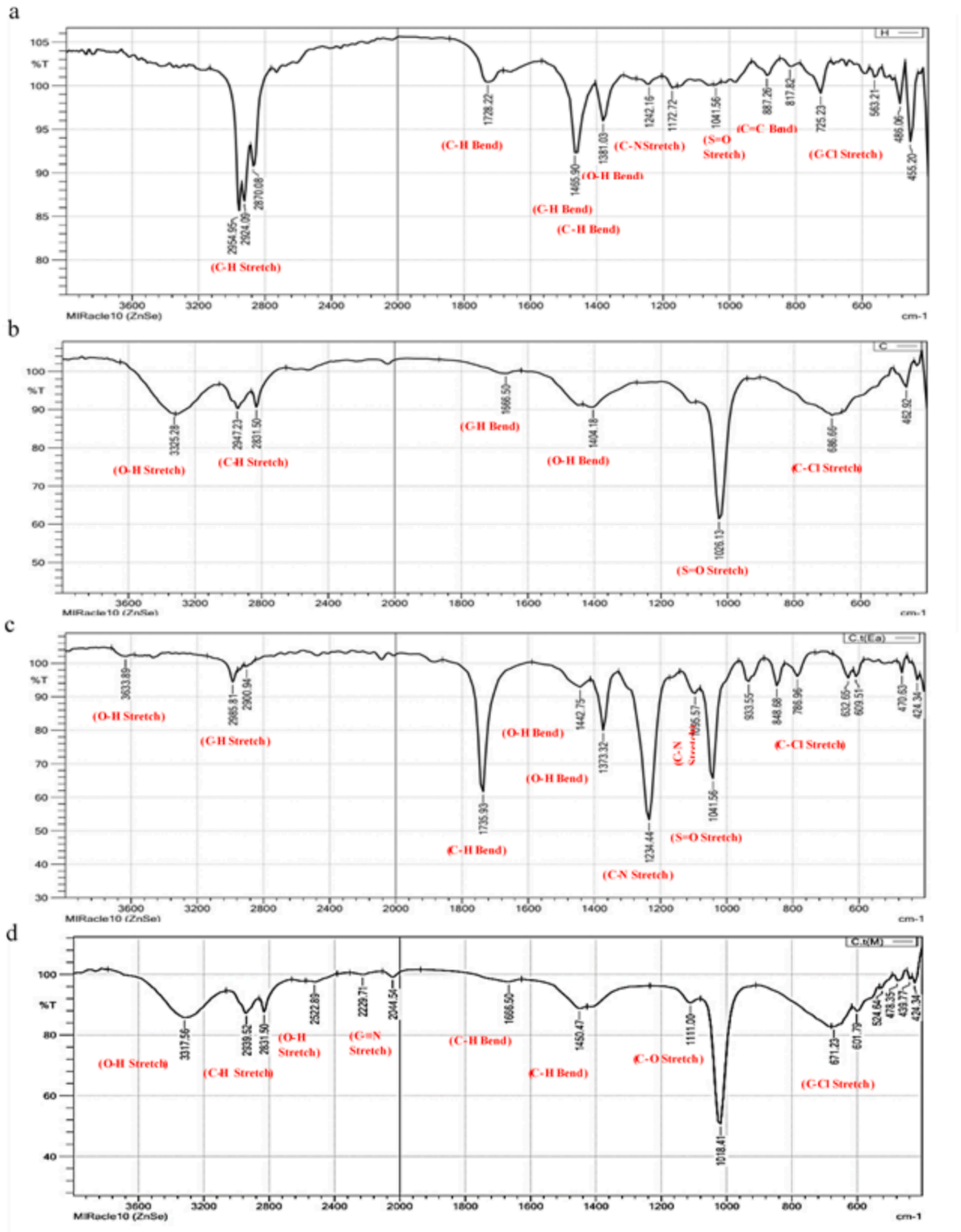

Fig. 2. Infrared spectra with Fourier transform in the fingerprint region of **a)** Hexane extract, b) Chloroform extract, c) Ethyl acetate extract and d) Methanol extract of *Cynanchum tunicatum.*

ergosta-8 (0.430 %), Pregn-5-en-20-one, 12-(acetyloxy)-3,8,14 (0.136 %), Cholesta-22,24-dien-5-ol, 4,4-dimethyl- (3.482 %), Lupeol (13.061 %), Megastigmatrienone (0.116 %), Lupenone (0.306 %), Beta −Amyrin (10.614 %), 17-(1,5-Dimethyl-3-phenylthiohex-4-enyl) (0.593 %) and Phytol (1.455 %). In Table 6 represents the compound name, molecular formula, retention time, peak area, functional group and reported medicinal applications for methanol extract of *C. tunicatum*. The structure of these metabolites was represented in Fig. 3. GC–MS analysis of *C. acutum* latex observed the chemical constituents such as lupeol, hexadecanoic acid, neophytadiene, octadecanoic acid and phytol were found with percentages of 15.36 %, 10.72 %, 9.15 %, 8.78 %, and 6.51 %, respectively (Soliman et al., 2022).

The GC–MS analysis of *Aristolochia tagala* leaf extracts revealed the presence of 42 compounds across various solvents such as petroleum

Table 6
Distribution of the identified metabolites through GC–MS in methanolic extracts of *Cynanchum tunicatum*.

Group	Compound name	Molecular formula	Retention time (min)	Peak Area (%)	Medical applications
Alkene	1-Hexacosene	$C_{26}H_{52}$	34.306	0.145	Antibacterial (Rani et al., 2019)
Cholestanoid	17-(1,5- Dimethylhexyl)-10,13-dimethyl-2	$C_{27}H_{46}O$	38.780	0.18	Antioxidant activity (Nyalo et al., 2023)
Dioxolanes	1,3-Dioxolane, 4-methyl-2-pentadecyl	$C_{21}H_{42}O_2$	3.104	0.382	Antimicrobial activity (Küçük et al., 2011)
Diosgenin	7-Dehydrodiosgenin	$C_{27}H_{40}O_3$	37.654	0.155	Antioxidant, Anti-inflammatory, Anticancer (Semwal et al., 2022)
Fatty acid	Methyl Palmitoleate	$C_{17}H_{32}O_2$	14.929	0.119	–
	Palmitic acid	$C_{16}H_{32}O_2$	25.998	0.988	Anticancer, Cardiovascular disease (Mancini et al., 2015)
	Methyl (Z)- 5,11,14,17- eicosatetraenoate	$C_{21}H_{34}O_2$	17.383	0.130	–
	Octadecanoic acid	$C_{18}H_{36}O_2$	18.714	0.751	Hypocholesterolemic activity (Selvaraju et al., 2021)
	Myristic acid	$C_{14}H_{28}O_2$	12.490	0.141	Larvicidal and Repellent activity (Chen et al., 2019)
	Methyl linoleate	$C_{19}H_{34}O_2$	17.266	0.147	Antioxidant activity (Davey et al., 2000)
L-Ascorbic acid	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	15.336	8.129	Antioxidant activity (Babouongolo et al., 2021)
Phytosterol	17-(1,5-Dimethyl-3-phenylthiohex-4-enyl) −4,4,10,13,14-pentamethyl 2,3,4,5,6,7,10,11,12-	$C_{36}H_{54}OS$	47.067	0.593	Anticancer (Suttiarporn et al., 2015)
	Campesterol	$C_{28}H_{48}O$	41.252	5.243	Anti-inflammatory and Cytotoxic activity (Bagewadi et al., 2019)
Phenol	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_3$	11.909	0.108	Antioxidant activity (Molan et al., 2012)
Steroid	14-Dimethyl-5 alpha-ergosta-8	$C_{30}H_{50}O$	42.870	0.430	Anti-inflammatory, Anticancer (Rasheed & Qasim, 2013)
	Pregn-5-en-20-one,12-(acetyloxy)-3,8,14	$C_{23}H_{34}O_6$	38.302	0.136	–
Terpenoid	Cholesta-22,24-dien-5-ol,4,4-dimethyl-	$C_{29}H_{48}O$	41.877	3.482	–
	Lupeol	$C_{30}H_{50}O$	46.326	13.061	Anti-inflammatory (Tsai et al., 2016)
	Megastigmatrienone	$C_{13}H_{18}O$	11.065	0.116	Antioxidant activity (Kyslychenko et al., 2010)
	Lupenone	$C_{30}H_{48}O$	45.271	0.306	Anti-inflammatory (Xu et al., 2020)
	Beta-Amyrin	$C_{30}H_{50}O$	44.767	10.614	Antibacterial activity, Anti-inflammatory activity (Dash et al., 2023)
	Phytol	$C_{20}H_{40}O$	17.572	1.455	Anti-inflammatory, Antimicrobial activity (Bagewadi et al., 2019)

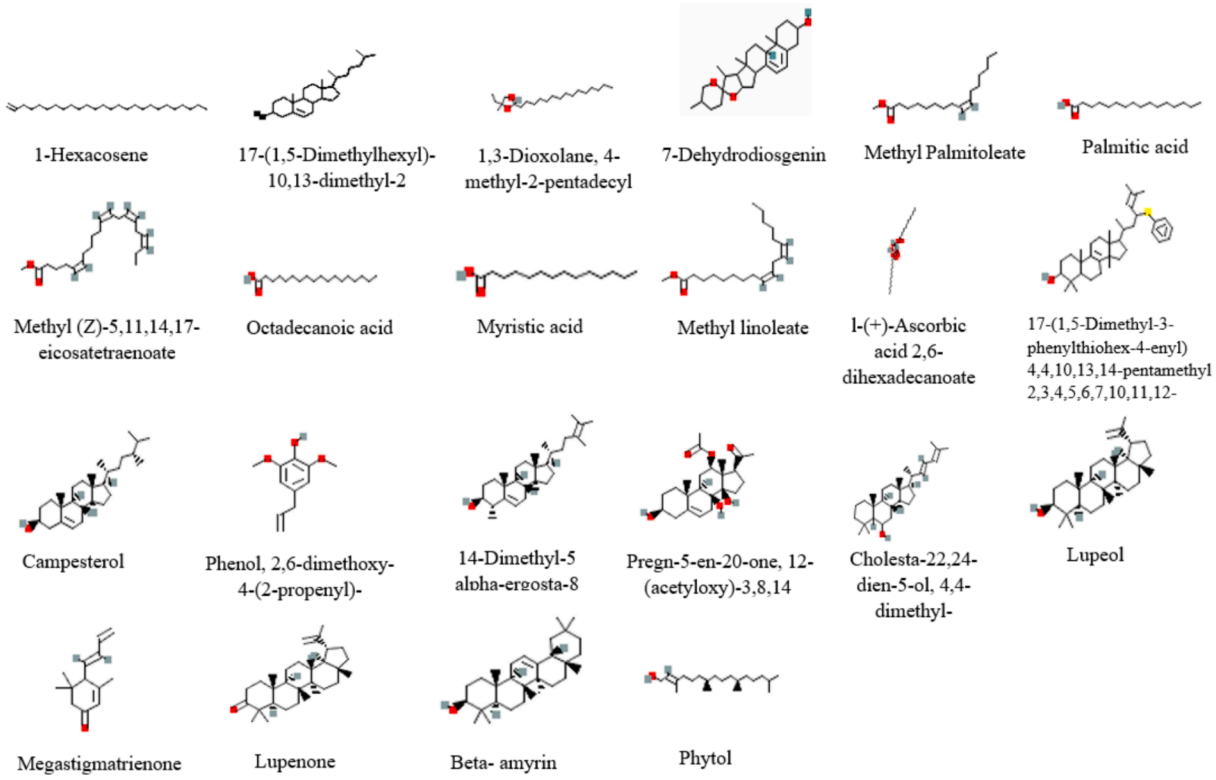

Fig. 3. Structure of the bioactive compounds obtained in the methanolic extract of *Cynanchum tunicatum* through GC–MS analysis.

ether, chloroform, ethyl acetate, methanol, and hydro-alcoholic (Mariyammal et al., 2023). Olivia *et al.*, utilized GC–MS analysis to identify twenty-three bioactive compounds in the hydromethanolic fraction of *Hibiscus asper* leaves such as 9,12,15-octadecatrien-1-ol, n-Hexadecanoic acid, octadecatrienol acid, methyl palmitate and phytol were significant phytoconstituents (Olivia et al., 2021). Lupeol and phytosterols possess various properties like antifungal, anti-inflammatory, antibacterial, anti-tumor, antioxidant and anti-ulcerative effects which plays their multifunctional biological roles (Ito et al., 2017). The GC–MS analyses showed preliminary insights into the bioactive components and evidenced the pharmaceutical applications for further research and drug discovery.

3.6. HPTLC profiling

In *C. tunicatum* the secondary metabolites were separated on HPTLC plates using various mobile phases such as Ethyl acetate: Methanol: Water (20:3:2), Ethyl acetate: Methanol: Formic acid: Water (20:3:1:2), Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) and Toluene: Acetone: formic acid (4.5:4.5:1) for alkaloid, flavonoid, phenols and terpenoids respectively.

HPTLC Fingerprinting of alkaloid evidenced eight spots with their corresponding ascending order of R_f value of 0.032, 0.224, 0.302, 0.474, 0.597, 0.669,0.776, 0.976 in 2.5 µL of methanolic extract. Spot 1, 2, 3, and 5 were identified as strychnine (Anti-inflammatory, anticancer, hepatoprotective, antioxidant, cardio protective, antidepressant, antidote for snakebite), colchicine (decreases inflammatory cytokines) (Senguttuvan & Subramaniam, 2016). While in flavonoid showed eight bands in 2.5 µL of methanolic extract with corresponding R_f values were 0.048, 0.082, 0.310, 0.456, 0.684, 0.813, 0.890 and 0.966. Whereas in 5 µL of extracts, 6 bands were noted, and its corresponding R_f values were 0.076, 0.460, 0.703, 0.834, 0.900, 0.971.

Phenol showed three bands with respective R_f values were 0.031, 0.258, 0.813 in 2.5 µL of methanol extracts and eight bands formed with corresponding R_f values were 0.032, 0.090, 0.227, 0.448, 0.489, 0.785, 0.850, 0.961 in 5 µL of extract. In terpenoid, nine bands have appeared with the corresponding R_f values were 0.045, 0.113, 0.173, 0.192, 0.285, 0.398, 0.463, 0.553 and 0.629 in 2.5 µL of methanol extracts while in 5 µL of extract, ten bands with R_f values were 0.050, 0.118, 0.176, 0.285, 0.344, 0.402, 0.469, 0.561, 0.635 and 0.745 were obtained. The alkaloids, flavonoids, phenols and terpenoids were identified and quantified by comparing them with standards like colchicine, quercetin, gallic acid and oleanolic acid, respectively (Fig. 4). These bioactive components were reported strong antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, anti-cardiovascular disease, antihemostatic, and chemopreventive activities (Deepika & Maurya, 2022). The metabolic screening showed that the primary constituents of the *C. tunicatum* extract comprised alkaloids, flavonoids, phenolic and terpenoids compounds.

4. Conclusion

In conclusion, the lacuna of requisite research in phytoconstituents of *Cynanchum tunicatum* influenced the spectrum of secondary metabolites, such as alkaloids, flavonoids, terpenoids and phenolic compounds were identified. In GCMS analysis methanolic extract of *C. tunicatum* showed 22 compounds were used for various therapeutic applications such as hypocholesterolemia, cancer, cardiovascular diseases, antiulcerative, antimicrobial, larvicidal and repellent activity. The phytochemicals identified using HPTLC fingerprinting were greatly beneficial for anti-inflammatory, anticancer, hepatoprotective, antioxidant, cardio protective, antidepressant, antidote for snakebite, and decreases inflammatory cytokines. Methanol extract of *C. tunicatum* evidenced potential in antioxidant and anti-inflammatory activities than the other

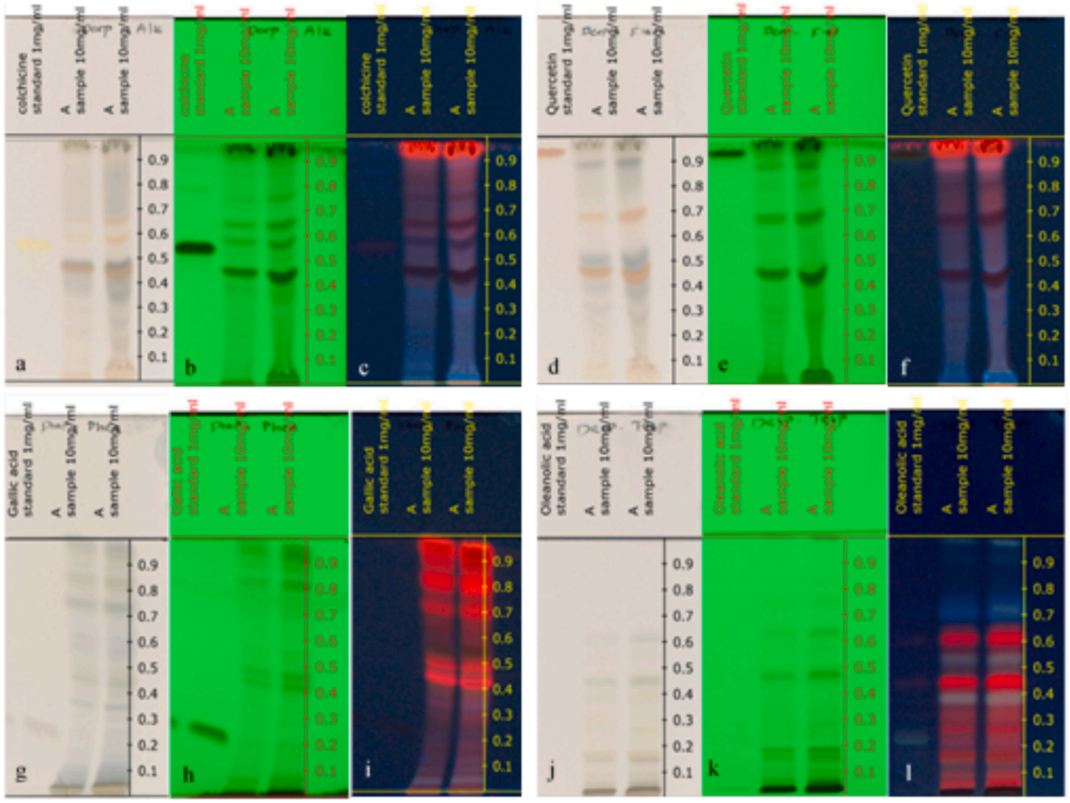

Fig. 4. TLC Profiles of *Cynanchum tunicatum.* a) Alkaloid at visible light b) Alkaloid at 366 nm c) Alkaloid at 254 nm d) Flavonoid at visible light e) Flavonoid at 366 nm f) Flavonoid at 254 nm g) Phenol at visible light h) Phenol at 366 nm i) Phenol at 254 nm j) Terpenoid at visible light k) Terpenoid at 366 nm l) Terpenoid at 254 nm.

extracts. Overall, the findings of this study of *C. tunicatum* as the major source of bioactive compounds with promising pharmacological activities suggested for further drug discovery from these identified compounds.

CRediT authorship contribution statement

Deepika Krishnamoorthy: Writing – original draft, Methodology, Investigation. **Amutha Swaminathan:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Amal Mohamed AlGarawi:** Writing – review & editing, Validation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lavanya Nallasamy:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Girija Sangari Murugavelu:** Writing – review & editing, Formal analysis. **Swarna Lakshmi Selvaraj:** Writing – review & editing, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

I would like to thank the Department of Botany and CNR Rao Laboratory and Advanced Research Laboratory, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India. The authors extend their appreciation to the Researchers supporting project number (RSPD2024R931) King Saud University, Riyadh, Saudi Arabia.

Funding

This work was supported by the Researchers supporting project number (RSPD2024R931) King Saud University, Riyadh, Saudi Arabia.

References

Abdel-Azeem, A., Nada, A.A., O'Donovan, A., Thakur, V.K., Elkelish, A., 2020. Mycogenic silver nanoparticles from endophytic Trichoderma atroviride with antimicrobial activity. J. Renewable Mater. 8 (2), 171–185.
Ajanal, M., Gundkalle, M.B., Nayak, S.U., 2012. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Anc. Sci. Life 31 (4), 198.
Akgül, H., Mohammed, F.S., Kına, E., Uysal, İ., Sevindik, M., Doğan, M., 2022. Total antioxidant and oxidant status and DPPH free radical activity of Euphorbia eriophora. Turkish J. Agric.-Food Sci. Technol. 10 (2), 272–275.
Alcazar Magana, A., Wright, K., Vaswani, A., Caruso, M., Reed, R.L., Bailey, C.F., Nguyen, T., Gray, N.E., Soumyanath, A., Quinn, J., 2020. Integration of mass spectral fingerprinting analysis with precursor ion (MS1) quantification for the characterisation of botanical extracts: application to extracts of Centella asiatica (L.) Urban. Phytochem. Anal 31 (6), 722–738.
Archana, H., Bose, V.G., 2022. Evaluation of phytoconstituents from selected medicinal plants and its synergistic antimicrobial activity. Chemosphere 287, 132276.
Arya, M., Singh, B.R., Taj, G., 2022. Phytochemical screening and quantitative analysis of Cichorium intybus L. (Chicory) plants from region of Uttarakhand. Pharma Innovat. J. 11 (4), 230–235.
Babouongolo, S.-G., Nkounkou Loumpangou, C., Dao, E., Simon, V., Elouma Ndinga, A. M., Ouamba, J.-M., 2021. Variability in aromatic composition of different fruit parts of pseudospondias microcarpa (A. Rich) Engl from Congo. J. Essential Oil Bearing Plants 24 (3), 421–430.
Bagewadi, Z.K., Muddapur, U.M., Madiwal, S.S., Mulla, S.I., Khan, A., 2019. Biochemical and enzyme inhibitory attributes of methanolic leaf extract of Datura inoxia Mill. Environ. Sustainability 2, 75–87.
Bailly, C., Xiang, C., Zhang, J.-H., 2023. Traditional uses and phytochemical constituents of Cynanchum otophyllum CK Schneid (Qingyangshen). World J. Trad. Chinese Med. 9 (1), 1–7.
Bayliak, M.M., Burdyliuk, N.I., Lushchak, V.I., 2016. Effects of pH on antioxidant and prooxidant properties of common medicinal herbs. Open Life Sci. 11 (1), 298–307.
Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239 (1), 70–76.
Chandra, S., Chatterjee, P., Dey, P., Bhattacharya, S., 2012. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac. J. Trop. Biomed. 2 (1), S178–S180.
Chen, X., Zhao, X., Deng, Y., Bu, X., Ye, H., Guo, N., 2019. Antimicrobial potential of myristic acid against Listeria monocytogenes in milk. J. Antibiot. 72 (5), 298–305.
Dash, S., Bohidar, J., Das, C., Mohanty, A., Meher, A., Hota, R., 2023. Evaluation of anthelmintic activity and GC-MS characterization of Urochloa distachya (L.). Int. J. Pharm. Invest. 13 (2).
Davey, M.W., Montagu, M.V., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J., 2000. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 80 (7), 825–860.
Deepika, Maurya, P.K., 2022. Health benefits of quercetin in age-related diseases. Molecules 27 (8), 2498.
Ene-Obong, H., Onuoha, N., Aburime, L., Mbah, O., 2018. Chemical composition and antioxidant activities of some indigenous spices consumed in Nigeria. Food Chem. 238, 58–64.
Garg, P., Garg, R., 2019. Phytochemical screening and quantitative estimation of total flavonoids of Ocimum sanctum in different solvent extract. Pharma Innov J 8 (2), 16–21.
Han, L., Zhou, X., Yang, M., Zhou, L., Deng, X., Wei, S., Wang, W., Wang, Z., Qiao, X., Bai, C., 2018. Ethnobotany, phytochemistry and pharmacological effects of plants in genus Cynanchum Linn. (Asclepiadaceae). Molecules 23 (5), 1194.
Harborne, A., 1998. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science & Business Media.
Hatano, T., Kagawa, H., Yasahara, H., Okuda, T., 1988. The effect of extracts on DPPH radical was estimated according to the methanol. Food Chem 78, 347–354.
Hmidani, A., Bourkhis, B., Khouya, T., Ramchoun, M., Filali-Zegzouti, Y., Alem, C., 2020. Phenolic profile and anti-inflammatory activity of four Moroccan date (Phoenix dactylifera L.) seed varieties. Heliyon 6 (2), e03436.
Indumathi, C., Durgadevi, G., Nithyavani, S., Gayathri, P., 2014. Estimation of terpenoid content and its antimicrobial property in Enicostemma litorrale. Int J ChemTech Res 6 (9), 4264–4267.
Ito, M., Ishimaru, M., Shibata, T., Hatate, H., Tanaka, R., 2017. High-performance liquid chromatography with fluorescence detection for simultaneous analysis of phytosterols (stigmasterol, β-sitosterol, campesterol, ergosterol, and fucosterol) and cholesterol in plant foods. Food Anal. Methods 10, 2692–2699.
Karale, P., Dhawale, S., Karale, M., 2022. Quantitative phytochemical profile, antioxidant and lipase inhibitory potential of leaves of Momordica charantia L. and Psoralea corylifolia L. Indian J. Pharm. Sci. 84 (1), 189–196.
Küçük, H.B., Yusufoğlu, A., Mataracı, E., Döşler, S., 2011. Synthesis and biological activity of new 1, 3-dioxolanes as potential antibacterial and antifungal compounds. Molecules 16 (8), 6806–6815.
Kyslychenko, V., Karpiuk, U., Ia, D., Abu-Darwish, M.S., 2010. Phenolic compounds and terpenes in the green parts of Glycine hispida. Adv. Environ. Biol. 490–495.
Mancini, A., Imperlini, E., Nigro, E., Montagnese, C., Daniele, A., Orrù, S., Buono, P., 2015. Biological and nutritional properties of palm oil and palmitic acid: effects on health. Molecules 20 (9), 17339–17361.
Mariyammal, V., Sathiageetha, V., Amalraj, S., Gurav, S.S., Amiri-Ardekani, E., Jeeva, S., Ayyanar, M., 2023. Chemical profiling of Aristolochia tagala Cham. leaf extracts by GC-MS analysis and evaluation of its antibacterial activity. J. Indian Chem. Soc. 100 (1), 100807.
Molan, A.-L., Faraj, A.M., Mahdy, A.S., 2012. Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq. Phytopharmacology 2 (2), 224–233.
Nagalakshmi, R., Anand, S., Prakash, M., 2023. Qualitative and quantitative phytochemical screening of tinospora cordifolia (Willd.). J. Stress Physiol. Biochem. 19 (3), 170–177.
Noreen, H., Semmar, N., Farman, M., McCullagh, J.S., 2017. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. Asian Pac. J. Trop. Med. 10 (8), 792–801.
Nyalo, P., Omwenga, G., Ngugi, M., 2023. Quantitative phytochemical profile and in vitro antioxidant properties of ethyl acetate extracts of Xerophyta spekei (Baker) and Grewia tembensis (Fresen). J. Evidence-Based Integrative Med. 28, 2515690X231165096.
Olivia, N.U., Goodness, U.C., Obinna, O.M., 2021. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves. Future J. Pharm. Sci. 7, 1–5.
Papakosta, V., Lopez-Costas, O., Isaksson, S., 2020. Multi-method (FTIR, XRD, PXRF) analysis of Ertebølle pottery ceramics from Scania, southern Sweden. Archaeometry 62 (4), 677–693.
Parvez, G., Sarker, R.K., 2021. Pharmacological potential of wood apple (Limonia acidissima): A Review. IJMFM AP 7 (2), 40–47.
Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269 (2), 337–341.
Raaman, N., 2006. Phytochemical techniques. New India Publishing.
Rahman, T.U., Khan, H., Liaqat, W., Zeb, M.A., 2022. Phytochemical screening, green synthesis of gold nanoparticles, and antibacterial activity using seeds extract of Ricinus communis L. Microsc. Res. Tech. 85 (1), 202–208.
Rani, R., Sharma, D., Chaturvedi, M., Yadav, J.P., 2019. Phytochemical analysis, antibacterial and antioxidant activity of Calotropis procera and Calotropis gigantea. Natural Products J. 9 (1), 47–60.
Rasheed, A., Qasim, M., 2013. A review of natural steroids and their applications. Int. J. Pharm. Sci. Res. 4 (2), 520.
Reignier, J., Méchin, F., Sarbu, A., 2021. Chemical gradients in PIR foams as probed by ATR-FTIR analysis and consequences on fire resistance. Polym. Test. 93, 106972.

Roaa, M., 2020. A review article: The importance of the major groups of plants secondary metabolism phenols, alkaloids, and terpenes. Int. J. Res. Appl. Sci. Biotechnol. (IJRASB) 7 (5), 354–358.
Saleem, A., Saleem, M., Akhtar, M.F., 2020. Antioxidant, anti-inflammatory and antiarthritic potential of Moringa oleifera Lam: An ethnomedicinal plant of Moringaceae family. S. Afr. J. Bot. 128, 246–256.
Selvaraju, R., Sakuntala, P., Jaleeli, K., 2021. GC–MS and FTIR analysis of chemical compounds in Ocimum gratissimum plant. Biophysics 66 (3), 401–408.
Semwal, P., Painuli, S., Abu-Izneid, T., Rauf, A., Sharma, A., Daştan, S. D., Kumar, M., Alshehri, M. M., Taheri, Y., Das, R., 2022. Diosgenin: an updated pharmacological review and therapeutic perspectives. *Oxidative Med. Cell. Longevity*, *2022*.
Senguttuvan, J., Subramaniam, P., 2016. HPTLC fingerprints of various secondary metabolites in the traditional medicinal herb Hypochaeris radicata L. J. Bot.
Shrivastava, A.K., Thapa, S., Shrestha, L., Mehta, R.K., Gupta, A., Koirala, N., 2021. Phytochemical screening and the effect of Trichosanthes dioica in high-fat diet induced atherosclerosis in Wistar rats. Food Front. 2 (4), 527–536.
Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic. 28 (1), 49–55.
Soliman, M.I., Mohammed, N.S., El-Sherbeny, G., Safhi, F.A., Alshamrani, S.M., Alyamani, A.A., Alharthi, B., Qahl, S.H., Al Kashgry, N.A.T., Abd-Ellatif, S., 2022. Antibacterial, antioxidant activities, GC-mass characterization, and cyto/genotoxicity effect of green synthesis of silver nanoparticles using latex of Cynanchum acutum L. Plants 12 (1), 172.
Sridharan, B., Yang, S.-Y., Li, J.-Y., Lee, M.-J., 2022. Effect of Cynanchum paniculatum (Bge.) Kitag. on various diseases: an overview of current research progress. Int. J. Appl. Sci. Eng. 19 (1), 1–11.
Suttiarporn, P., Chumpolsri, W., Mahatheeranont, S., Luangkamin, S., Teepsawang, S., Leardkamolkarn, V., 2015. Structures of phytosterols and triterpenoids with potential anti-cancer activity in bran of black non-glutinous rice. Nutrients 7 (3), 1672–1687.
Tsai, F.-S., Lin, L.-W., Wu, C.-R., 2016. Lupeol and its role in chronic diseases. Drug Discovery Mother Nature 145–175.
Vahur, S., Teearu, A., Peets, P., Joosu, L., Leito, I., 2016. ATR-FT-IR spectral collection of conservation materials in the extended region of 4000–80 cm–1. Anal. Bioanal. Chem. 408, 3373–3379.
Valdez, C.A., Leif, R.N., Hok, S., Alcaraz, A., 2018. Assessing the reliability of the NIST library during routine GC-MS analyses: Structure and spectral data corroboration for 5, 5-diphenyl-1, 3-dioxolan-4-one during a recent OPCW proficiency test. J. Mass Spectrom. 53 (LLNL-JRNL-745368).
Wang, L., Cai, F., Zhao, W., Tian, J., Kong, D., Sun, X., Liu, Q., Chen, Y., An, Y., Wang, F., 2021. Cynanchum auriculatum Royle ex Wight., Cynanchum bungei Decne. and Cynanchum wilfordii (Maxim.) Hemsl.: Current Research and Prospects. Molecules 26 (23), 7065.
Xu, F., Yang, L., Huang, X., Liang, Y., Wang, X., Wu, H., 2020. Lupenone is a good anti-inflammatory compound based on the network pharmacology. Mol. Divers. 24, 21–30.
Yesmin, S., Paul, A., Naz, T., Rahman, A.A., Akhter, S.F., Wahed, M.I.I., Emran, T.B., Siddiqui, S.A., 2020. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clin. Phytosci. 6, 1–10.
Zhang, Y., Hu, C., 2020. Anticancer activity of bisindole alkaloids derived from natural sources and synthetic bisindole hybrids. Arch. Pharm. 353 (9), 2000092.